Protocol for Relative Promoter Unit Measurement Using FACS

1. Restreak the plates two days before measurement

Construct	Plasmid	Strain
E0240	pSB3K3	DH10B
I20260	pSB3K3	DH10B
kdpFp (T>G)	pSB3K3	DH10B

2. Pick a colony from each plate to start overnight culture in 2mL of K115 minimal medium at 37 in 12mL falcon tubes.
3. Prepare the stock of media of specific K^+ concentration:K0, K0.0125, K0.025, K0.05, K0.1, K0.2, K0.4 in new falcons and then aliquot1 mL into Corning® 96 well storage system storage block, 2 mL, V-bottom.
4. Wash the cell three times with 0.8% NaCl solution (2mL each). In between each washing step, spin the cells with 3000rpm at 4 for 3 minutes. After washing, resuspend the cells in 700L of fresh K0 medium.
5. Take out 200L of washed cells to check OD595 and then dilute all the suspensions to around 1 (OD595).
6. Take out 25L (1/40 of the medium) of washed cells to mix with K minimal medium of different K concentration in the storage block.
7. Incubate the culture in 37 for 1-2 hours until it reaches the mid-exponential phase (OD600: 0.3-0.5)
8. Take out 200L of the culture from the storage block and put into a micro test plate 96 well flat-bottom. MeasureOD595 using Envision Multilabel Reader every 15 minutes for three times in total. In between measurements, keep incubating the cells in 37 while shaking.
9. For one of the three time points, fix the cells after the OD measurement by mixing 200L of cell culture sample with 200L of formaldehyde. Green fluorescence level for the whole cell population was measured by FACS and the geometric mean was treated as the average green fluorescence level for individual cells.
10. Repeat step 1-9 3 more times with other colonies.

Data Processing for Relative Promoter Unit Measurement

1. Average green fluorescence level of individual cell was obtained from the geometric mean of FACS reading. The fluorescence given by construct DH10B/pSB3K3-BBa_E0240 was regarded as the background so after subtracting the value of this construct in different media, average green fluorescence level of individual cell for experimental and constitutive constructs were obtained.

2. Growth rate was approximated by the slope of OD595 over time. The average of three blank K0 media was used to subtract the background absorbance during the process.

3. $[F]_{cell}$ stands for average green fluorescence level of individual cell, stands for the growth rate, and stands for the experimental promoter. Following this equation, relative promoter unit for the experimental promoter can be calculated.

References:

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